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Characterization of Recombinant XPF Endonuclease and XPF-ERCC1

Complex; M. Hwang, T. Bessho*, A. Sancar*, K. Brookman, L. Thompson, S. McCutchen-Maloney, and M. P. Thelen; Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, California; and * Department of Biochemistry, University of North Carolina, Chapel Hill.

Among the nucleotide excision repair genes, *XPF* and *ERCC1* are uniquely involved in removing DNA interstrand cross-linking damage. The encoded XPF protein sequence is homologous to proteins from the yeasts and drosophila that are each characterized by a dual function in excision repair and in genetic recombination. This functional duality is mirrored by the ERCC1 homologs, and reflects the complex formed by the XPF and ERCC1 proteins. During excision repair, this complex makes a single incision 5'-ward of the lesion¹. The precise contribution to this endonuclease activity by the individual XPF and ERCC1 partners is not known. To address this and related questions concerning recombination, we first used our *ERCC4(XPF)* cDNA² to produce part or all of the XPF protein in *E. coli*, and raised several polyclonal and monoclonal antibodies that were targeted to different regions of the protein. For subsequent biochemical studies, XPF was generated as a histidine-tagged, thioredoxin (trx) fusion protein ("trxXPF"). The 10kDa trxA sequence fused to the N-terminus of XPF resulted in an increase in the expression of soluble protein. Overexpression of both ERCC1 and the his-tagged trxXPF from the same plasmid generated the complex that bound to a Ni²⁺-affinity column. Using this and subsequent chromatographic steps, trxXPF and the trxXPF-ERCC1 complex were purified. When tested for endonucleolytic nicking of supercoiled DNA, both purified protein samples exhibited activity that was specifically stimulated by addition of anti-XPF antibodies, possibly due to disaggregation or stabilization of the active form of XPF. Thus the XPF protein appears to be the catalytic component of the endonuclease complex. For studies on interactions with other repair proteins, XPF-ERCC1 co-expressed in Sf21 insect cells from a baculovirus vector was purified and tested for activity³. The purified complex contained the anticipated 5'-junction specific endonuclease activity that was stimulated through a direct interaction between XPF and replication protein A (RPA). Excision nuclease activity was reconstituted in a mixture of 5 repair factors (XPA, XPC, XPG, TFIIH, RPA) that was completed by addition of the recombinant XPF-ERCC1. (Work was funded by NIH grant GM52120 to MPT; research was performed under the auspices of the U.S. DOE by LLNL under contract No. W-7405-ENG-48)

1 Park et al, J. Biol. Chem. 270, 22657-60, 1995.

- 2 Brookman et al, Mol. Cell. Biol. 16, 6553-6562, 1996.
- 3 Bessho et al, J. Biol. Chem., in press.